

WHAT IS CLAIMED IS:

1. A method for regenerating transgenic plants of pine of the genus *Pinus* subgenus *Pinus* which comprises:

5 subjecting pine cells to *Agrobacterium* infection for *Agrobacterium* transformation;

minimizing damage to cells subsequent to *Agrobacterium* infection;

rapidly selecting transformed cells;

culturing said transformed cells to produce transgenic somatic embryos; and

germinating said transgenic somatic embryos to produce transgenic plants.

- 10 2. The method of claim 1, wherein said damage to cells is minimized by:

(a) resuspending cells having been subjected to transformation in a liquid wash medium;

(b) agitating said cells in said liquid wash medium;

(c) recovering washed, transformed cells with minimal damage.

- 15 3. The method of claim 2, wherein pine cells are plated onto a support membrane prior to subjecting to *Agrobacterium* transformation.

- 20 4. The method of claim 1, wherein said damage to cells is minimized by:

(a) plating pine cells on a support membrane;

(b) rinsing said cells using a liquid wash medium;

(c) recovering washed, transformed cells with minimal damage.

- 25 5. The method of claim 4, wherein pine cells are plated onto a support membrane prior to subjecting to *Agrobacterium* transformation.

- 30 6. The method of claim 4, wherein pine cells are plated onto a support membrane subsequent to subjecting to *Agrobacterium* transformation.

7. The method of claim 4, wherein steps (b) and (c) are repeated until *Agrobacterium* contamination is no longer detectable.

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8. The method of claim 7, wherein said steps are repeated between 2 and 10 times.
9. The method of claim 4, wherein each wash is carried out for a duration sufficient to expose all the cells to the wash medium without interfering with subsequent growth of the plant cells.
10. The method of claim 9, wherein each wash is carried out for between half an hour to overnight in duration.
11. The method of claim 4, wherein said support membrane is prepared from a material selected from the group consisting of polyester, polypropylene and a liquid permeable fluoropolymer fabric.
12. The method of claim 1, wherein said rapid selection is performed by culturing cells which have been subjected to transformation on a support membrane placed over a gel medium; contacting said cells with a selection agent; and selecting transformed cells.
13. The method of claim 12, wherein said selection agent is contained in said gel medium.
14. The method of claim 12, wherein said selection agent is contained in a layer and said support membrane is placed over said layer which is placed on said gel medium.
15. The method of claim 14, wherein said layer is a thin film of liquid medium.
16. The method of claim 14, wherein said layer is a thin film of gelled medium.
17. The method of claim 14, wherein said layer is a filter paper with a liquid medium absorbed therein.

18. The method of claim 12, wherein said support membrane is prepared from a material selected from the group consisting of polyester, polypropylene and a liquid permeable fluoropolymer fabric.

5 19. The method of claim 1 which further comprises the eradication of *Agrobacterium*.

20. The method of claim 19, wherein said eradication is performed by:
culturing cells which have been subjected to transformation on a support membrane over a layer containing an eradicant, said layer in or positioned over a gel medium; and
recovering cells from which said *Agrobacterium* has been eradicated.

10 21. The method of claim 20, wherein said layer is a thin film of liquid medium.

5 22. The method of claim 20, wherein said layer is a thin film of gelled medium.

23. The method of claim 20, wherein said layer is a filter paper with a liquid medium absorbed therein.

20 24. The method of claim 20, wherein said support membrane is prepared from a material selected from the group consisting of polyester, polypropylene and a liquid permeable fluoropolymer fabric.

25. A method for regenerating transgenic plants of pine of the genus *Pinus* subgenus *Pinus* which comprises:

subjecting pine cells to *Agrobacterium* infection for *Agrobacterium* transformation;

eradicating *Agrobacterium*;

minimizing damage to cells during and subsequent to *Agrobacterium* eradication;

rapidly selecting transformed cells;

culturing said transformed cells to produce transgenic somatic embryos; and

germinating said transgenic somatic embryos to produce transgenic plants.

26. The method of claim 25, wherein said damage to cells is minimized by:
- (a) resuspending cells having been subjected to transformation in a liquid wash medium;
 - (b) agitating said cells in said liquid wash medium;
 - (c) recovering washed, transformed cells with minimal damage.
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27. The method of claim 26, wherein pine cells are plated onto a support membrane prior to subjecting to *Agrobacterium* transformation.
- 10 28. The method of claim 26, wherein said rapid selection is performed by
- culturing cells which have been subjected to transformation on a support membrane placed over a gel medium;
 - contacting said cells with a selection agent; and
 - selecting transformed cells.
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29. The method of claim 26, wherein said eradication is performed by:
- culturing cells which have been subjected to transformation on a support membrane over a layer containing an eradicant, said layer in or positioned over a gel medium; and
 - recovering cells from which said *Agrobacterium* has been eradicated.
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30. The method of claim 28, wherein said eradication is performed by:
- culturing cells which have been subjected to transformation on a support membrane over a layer containing an eradicant, said layer in or positioned over a gel medium; and
 - recovering cells from which said *Agrobacterium* has been eradicated.
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31. The method of claim 25, wherein said damage to cells is minimized by:
- (a) plating pine cells on a support membrane;
 - (b) rinsing said cells using a liquid wash medium;
 - (c) recovering washed, transformed cells with minimal damage.
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32. The method of claim 31, wherein pine cells are plated onto a support membrane prior to subjecting to *Agrobacterium* transformation.

5 33. The method of claim 31, wherein pine cells are plated onto a support membrane subsequent to subjecting to *Agrobacterium* transformation.

34. The method of claim 31, wherein said rapid selection is performed by
culturing cells which have been subjected to transformation on a support
membrane placed over a gel medium;
10 contacting said cells with a selection agent; and
selecting transformed cells.

15 35. The method of claim 31, wherein said eradication is performed by:
culturing cells which have been subjected to transformation on a support
membrane over a layer containing an eradicant, said layer in or positioned over a gel
medium; and
recovering cells from which said *Agrobacterium* has been eradicated.

20 36. The method of claim 34, wherein said eradication is performed by:
culturing cells which have been subjected to transformation on a support
membrane over a layer containing an eradicant, said layer in or positioned over a gel
medium; and
recovering cells from which said *Agrobacterium* has been eradicated.

25 37. The method of claim 25, wherein said rapid selection is performed by
culturing cells which have been subjected to transformation on a support
membrane placed over a gel medium;
contacting said cells with a selection agent; and
selecting transformed cells.

30 38. The method of claim 25, wherein said eradication is performed by:

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culturing cells which have been subjected to transformation on a support membrane over a layer containing an eradicant, said layer in or positioned over a gel medium; and

recovering cells from which said *Agrobacterium* has been eradicated.

- 5 39. A method for minimizing damage to transformed cells of pine of the genus *Pinus* subgenus *Pinus* following infection by *Agrobacterium* for *Agrobacterium* transformation which comprises:
- 10 (a) washing transformed cells in a liquid wash medium;
 - (b) plating said cells on a support membrane;
 - (c) resuspending said cells in a liquid wash medium; and
 - (d) recovering washed, transformed cells with minimal physical damage.
- 15 40. The method of claim 39, wherein (i) cells are plated onto a support membrane and (ii) said cells are transformed prior to step (a).
- 20 41. The method of claim 39, wherein steps (b) and (c) are repeated until *Agrobacterium* contamination is no longer detectable.
- 25 42. The method of claim 41, wherein said steps are repeated between 2 and 10 times.
43. The method of claim 39 wherein each wash is carried out for a duration sufficient to expose all the cells to the wash medium without interfering with subsequent growth of the pine cells.
44. The method of claim 43, wherein each wash is carried out for between half an hour to overnight in duration.
- 30 45. The method of claim 39, wherein said support membrane is prepared from a material selected from the group consisting of polyester, polypropylene and a liquid permeable fluoropolymer fabric.

46. A method for pine cell tissue culture which comprises culturing pine cells on a support membrane placed over a gel medium.

5 47. The method of claim 46, wherein said support membrane is placed over a layer containing one or more culture components, said layer is positioned on said gel medium.

48. The method of claim 46, wherein said cells are plated onto said support membrane prior to culturing.

10 49. The method of claim 47, wherein said layer is a thin film of liquid medium.

50. The method of claim 47, wherein said layer is a filter paper with a liquid medium absorbed therein.

15 51. The method of claim 46, wherein said support membrane is prepared from a material selected from the group consisting of polyester, polypropylene and a liquid permeable fluoropolymer fabric.

20 52. A method for selecting transformed cells of pine of the genus *Pinus* subgenus *Pinus* which comprises:

culturing cells which have been subjected to transformation on a support membrane placed over a gel medium;

contacting said cells with a selection agent; and

selecting transformed cells.

25 53. The method of claim 52, wherein said selection agent is contained in said gel medium.

54. The method of claim 52, wherein said selection agent is contained in a layer and said support membrane is placed over said layer which is positioned on said gel medium

30 55. The method of claim 54, wherein said layer is a thin film of liquid medium.

56. The method of claim 54, wherein said layer is a filter paper with a liquid medium absorbed therein.

5 57. The method of claim 52, wherein said support membrane is prepared from a material selected from the group consisting of polyester, polypropylene and a liquid permeable fluoropolymer.

10 58. A method for eradicating *Agrobacterium* from cells of pine of the genus *Pinus* subgenus *Pinus* which comprises:

culturing cells on a support membrane over a layer containing an eradicant, said layer positioned in or over a gel medium; and

recovering cells from which said *Agrobacterium* contaminant has been eradicated.

15 59. The method of claim 58, wherein said layer is a thin film of liquid medium.

60. The method of claim 58, wherein said layer is a thin film of gelled medium.

15 61. The method of claim 58, wherein said layer is a filter paper with a liquid medium absorbed therein.

20 62. The method of claim 58, wherein said support membrane is prepared from a material selected from the group consisting of polyester, polypropylene and a liquid permeable fluoropolymer fabric.

25 63. A transformed embryogenic culture prepared by the method of claim 39.

64. A transformed embryogenic culture prepared by the method of claim 52.

30 65. A transformed embryogenic culture prepared by the method of claim 58.

66. A transformed pine plant of the genus *Pinus* subgenus *Pinus* prepared by the method of claim 1.

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67. A transformed pine plant of the genus *Pinus* subgenus *Pinus* prepared by the method of
claim 2.

5 68. A transformed pine plant of the genus *Pinus* subgenus *Pinus* prepared by the method of
claim 4.

69. A transformed pine plant of the genus *Pinus* subgenus *Pinus* prepared by the method of
claim 12.

10 70. A transformed pine plant of the genus *Pinus* subgenus *Pinus* prepared by the method of
claim 19.

15 71. A transformed pine plant of the genus *Pinus* subgenus *Pinus* prepared by the method of
claim 25.

72. A transformed pine plant of the genus *Pinus* subgenus *Pinus* prepared by the method of
claim 26.

20 73. A transformed pine plant of the genus *Pinus* subgenus *Pinus* prepared by the method of
claim 28.

74. A transformed pine plant of the genus *Pinus* subgenus *Pinus* prepared by the method of
claim 29.

25 75. A transformed pine plant of the genus *Pinus* subgenus *Pinus* prepared by the method of
claim 30.

30 76. A transformed pine plant of the genus *Pinus* subgenus *Pinus* prepared by the method of
claim 31.

77. A transformed pine plant of the genus *Pinus* subgenus *Pinus* prepared by the method of
claim 34.

78. A transformed pine plant of the genus *Pinus* subgenus *Pinus* prepared by the method of claim 35.
- 5 79. A transformed pine plant of the genus *Pinus* subgenus *Pinus* prepared by the method of claim 36.
80. A transformed pine plant of the genus *Pinus* subgenus *Pinus* prepared by the method of claim 37.
- 10 81. A transformed pine plant of the genus *Pinus* subgenus *Pinus* prepared by the method of claim 38.

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